Short communication

In vitro propagation of *Drosera natalensis*

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The successful *in vitro* propagation of *Drosera natalensis* Diels. is described. Rapid clonal multiplication was achieved on modified Murashige and Skoog medium supplemented with various hormones. The production of buds on the surface of explant tissue, their subsequent subculture onto a series of defined media and the hardening off of the resultant plantlets are outlined.

Die suksesvolle *in vitro*-vermeerdering van *Drosera natalensis* Diels. word beskryf. Vinnige vermeerdering is verkry met 'n gemodifiseerde Murashige en Skoog-medium wat verskeie hormone bevat het. Die produksie van knoppe op die oppervlakte van eksplant-weefsel, hulle subkultuur op 'n reeks gedefinieerde media en die afharding van die gevormde plantjies word beskryf.

Keywords: *Drosera natalensis*, micropropagation, tissue culture

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Drosera is a carnivorous plant noted for having a number of phytotherapeutic properties. In Europe, certain of the plant's chemical constituents and tinctures have been used by allopaths and homeopaths in the treatment of bronchial infections (Denoël 1948). The active compound, plumbagin (5-hydroxy-2-methyl-1-4-naphthoquinone) an inhibitor of enzyme activity reacts readily with SH and NH_2 radicles. Its liposoluble properties suggest interesting therapeutic applications especially in the area of tuberculosis (Denoël 1948). Heise & Steenken (1941) and Lloyd & Middlebrook (1944) showed that this compound may have an inhibitory action on the growth of the tuberculosis bacterium. Zenk *et al.* (1969) list a number of species within the Droseraceae in which the naphthoquinone may be found. Frequent harvesting of natural populations in Europe have resulted in plants becoming increasingly scarce. This had led to an increased interest in new ways of propagating the plant. Bonnet *et al.* (1984) succeeded in propagating *D. rotundifolia* by multiplying and growing plants vegetatively from seeds germinated *in vitro*. Attempts to sterilize vegetative material from mature plants taken from natural populations proved unsuccessful since surface sterilization was ineffective or frequently killed the tissue. The objective of this investigation was to establish *in vitro* plantlets of *Drosera natalensis* Diels., a South African species, using mature explant material grown *in vivo*.

Mature *D. natalensis* plants were collected from the environs of Hammarsdale. This study investigated the totipotency of all the plant organs. Explants were derived from entire mature leaves (15 mm), young unopened shoots (5 mm) and flower buds (7 mm). Additional explant material included 10-mm lengths of flower stalk and root material. The material was surface sterilized in 1,5% sodium hypochloride (Table 1) and then washed three times in sterile distilled water. Thereafter the explants were placed on modified Murashige & Skoog (1962) medium, supplemented with the hormones benzyladenine (BA) and naphthalene acetic acid (NAA), for 60 days and then sequentially subcultured onto successive media as indicated in Table 2. All media were adjusted to pH 5,8 prior to autoclaving and were solidifed with 0,8% agar. The cultures were maintained in a growth room at 25 ± 2°C with a 16-h light:8-h dark cycle. Illumination was supplied by cool, white fluorescent tubes with a light intensity of 27 $\mu Em^{-2}S^{-1}$. Once formed, *in vitro* plantlets with well-developed roots (Figure 1) were transplanted into small pots containing a sterilized sand:peat moss (1:2) mixture. The pots were enclosed in plastic bags which contained a small volume of distilled water. After 10 days, the plastic bags were removed and the pots placed in a mist house. A final move to natural growing conditions after 3 weeks completed the hardening process.

Table 1 Sterilization of explants from different organs of *D. natalensis* using NaOCl (1,5%) as a sterilant

Explant source	Time of immersion (min)	Plants decontaminated and surviving (%)
Mature leaf	7,00	35
Young leaf	5,00	32
Flower stalk	3,25	32
Flower bud	3,25	90

Table 2 Outline of the culturing procedure for the *in vitro* propagation of *D. natalensis*

Procedure	Medium	Culture period (days)	Success rate (%)
Stage 1: production of buds	Sucrose 30 g l^{-1}; 1/5 MS + NAA 0,025 mg l^{-1}; BA 0,1 mg l^{-1}	40 – 60	35
Stage 2: production of shoots from buds	Sucrose 30 g l^{-1}; 1/5 MS + NAA 0,025 mg l^{-1}; BA 0,1 mg l^{-1}	80	100
Stage 3: induction of roots	Sucrose 30 g l^{-1}; 1/5 MS + NAA 0,1 mg l^{-1}; BA 0,0125 mg l^{-1}	100	100
Stage 4: hardening off of plantlets	Plantlets planted in sterile sand:peat moss (1:2)	140	80

Mature explants were successfully sterilized as indicated in Table 1. Higher concentrations of sterilant at reduced times of immersion invariably resulted in oxidation of the explant tissue. Decontamination of root explants proved difficult because of ineffective sterilization of internal pathogens.

Benzyladenine applied at 0,1 mg l^{-1} and NAA at 0,025 mg l^{-1} resulted in the formation of buds (Figure 2) on all the explants (Table 2). On average 20 newly formed buds were produced on each mature leaf explant. Flower buds and flower stalks on the other hand produced fewer buds which also had a reduced rate of development. Differentiation into

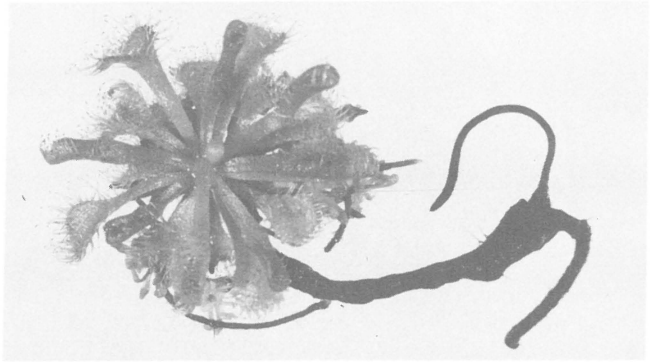

Figure 1 Juvenile plantlet of *Drosera natalensis* cultured *in vitro*.

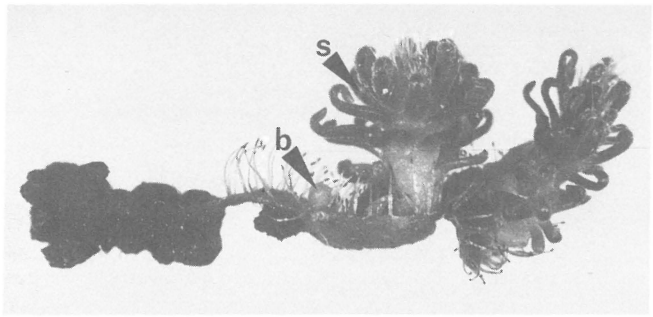

Figure 2 Newly formed bud (b) on the adaxial surface of a young leaf explant of *Drosera natalensis*. Each bud developed into a rosette of shoots (s) within 3 weeks in culture.

shoots was achieved by transferring the buds onto the stage 2 medium (stage 2, Table 2) recommended for bud proliferation. A high cytokinin to auxin ratio (0,1 mg l^{-1} BA: 0,025 mg l^{-1} NAA) resulted in embryogenesis regardless of explant source. Root induction was enhanced at 0,1 mg l^{-1} NAA and 0,0125 mg l^{-1} BA. Using this technique, about 1 500 plantlets could be obtained within 8 months from a single mature leaf explant.

Acknowledgements

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References

BONNET, M., COUMANS, M., RAMAUT, J.L. & GASPAR, T. 1984. Vegetative multiplication *in vitro* of the sundew *Drosera rotundifolia*. *Archs int. Physiol. Biochim.* 9: 16–17.

DENOËL, A. 1948. Determination de l'activité des *Droseras* indigenes et de leurs teintures. *J. Pharm. Belgique* 1–2: 3–19.

HEISE, F.H. & STEENKEN, W. 1941. Growth and virulence of tubercle bacilli. *Am. Rev. Tuberc.* 44: 635–636.

LLOYD, J.B. & MIDDLEBROOK, G. 1944. Bacteriostatic activity of some new derivatives of diaminodiphenylsulfone and naphthoquinones against the tubercle bacillus. *Am. Rev. Tuberc.* 49: 539–542.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Pl.* 15: 473–497.

ZENK, M.H., FURBRINGER, M. & STEGLICH, W. 1969. Occurrence and distribution of 7-methyljuglone and plumbagine in the Droseraceae. *Phytochem.* 8: 2199.

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